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# STEROLS AND ALKENONES OF *ISOCHRYSIS*

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**Key Word Index**—*Isochrysis*; Prymnesiophyceae; brassicasterol; cholesterol; sterols; alkenones; $\Delta^5$-sterols; dihydroxysterols; epibrassicasterol; chemical taxonomy.

**Abstract**—Sterols were identified and quantified in six marine microalgal strains identified as belonging to the prymnesiophyte genus *Isochrysis*; several of these strains are in wide use in the commerical mariculture industry. One strain contained only cholesterol, and another contained a complex mixture of $\Delta^5$-sterols and dihydroxysterols. Two strains contained brassicasterol and two others contained epibrassicasterol as the principal sterol. The strains containing brassicasterol or epibrassicasterol also contained long chain alkenones characteristic of some members of the Prymnesiophyceae; whereas, those without brassicasterol or epibrassicasterol contained no long chain alkenones. These qualitative biochemical differences appear to have taxonomic significance and may be important in the value of these algae as live feeds for rearing marine invertebrates.

## INTRODUCTION

Since the 1950s, a number of prymnesiophyte flagellates identified as *Isochrysis* have been cultured and exchanged between academic, commercial and government facilities. The type species, *I. galbana*, was described from cultured material (Flagellate I) by Parke [1] and amended by Green and Pienaar [2]. Distinctive features of the species include a size of 5–7 $\mu$m, a variable-shaped, wall-less cell morphology and two equal, apical flagellae astride a distinctive, abbreviated haptonema. In addition to the type strain, several other strains identified as *Isochrysis* have proven to be cultured readily, are small enough to be ingested by larval stages of invertebrates, are digestible by virtue of lacking a cell wall, and are capable of supporting growth of a number of invertebrates of commercial value, especially bivalve mollusks [3].

Comparative studies have shown differences between *Isochrysis* strains isolated at different times and from different places in temperature tolerance [4], fatty acid profile [5, 6], amino acids and sugars [7], and nutritional value to invertebrates [5, 8]. Despite this evidence of differences between *Isochrysis* strains, they often are viewed as essentially interchangeable as aquaculture feeds.

As sources of nutrition for secondary consumer species, e.g. commercially reared bivalve mollusks, *Isochrysis* strains are of interest from a biochemical standpoint. The importance of gross biochemical composition and essential fatty acids in bivalve nutrition has been established [9–12]; however, a complete characterization of the optimal diet for rearing bivalves has not been accomplished. Recently, the growth rate of juvenile, post-set oysters has been correlated positively with the quantity and type of sterol in the diet [13]. We therefore were interested in determining the sterol composition of cultured strains identified as belonging to the genus *Isochrysis*.

The first reports of sterols from *Isochrysis* were from *Isochrysis* sp. (T-ISO) [14], and *I. galbana* [15] from the Plymouth culture collection (strain designation not listed). Each report listed 24-methyl-22-dehydrocholesterol as the principal sterol. Subsequently, others have identified 24-methyl-22-dehydrocholesterol from *I. galbana* [16] and from *I. galbana* Parke, Plymouth 'I' isolate (the type culture), and *Isochrysis* spp. Plymouth isolates 507 and 506a [17]. The configuration of C-24 was not reported until Goad *et al.* [18], using 220 MHz NMR, determined that *I. galbana* (Plymouth I strain) contained the 24$\alpha$-methyl isomer, epibrassicasterol. Gladu *et al.* [19], however, using HPLC, determined that *Isochrysis* sp. strains T-ISO, and C-ISO contained the C-24$\beta$-methyl isomer brassicasterol. Both quantity and sterol-type appear to be important in the diet of the oyster, therefore we believe it was important to analyse the sterols of several *Isochrysis* strains quantitatively and to determine the C-24 orientation of strains not examined previously.

## RESULTS AND DISCUSSION

Sterols were analysed in six cultured strains of *Isochrysis* from various culture collections (see Experimental). Four of the six strains contained primarily one

Table 1. Sterols of *Isochrysis* sp.

| Strain | $\mu g\,g^{-1}$ dry wt | $fg\,cell^{-1}$ | Sterol composition* CHOL | BRASS | EPI | 24MC | 24EC-5 22 | 24EC | MPAV |
|---|---|---|---|---|---|---|---|---|---|
| *Isochrysis* | | | | | | | | | |
| *galbana* BL-ISO | 0.35 | 5 | | | 99 | | | | |
| I. sp. LB-1292 | 0.55 | 6 | | | 99 | | | | |
| I. sp. T-ISO | 0.95 | 31 | | 99 | | | | | |
| I. sp. C-ISO | 0.70 | 21 | | 99 | | | | | |
| I. sp. ISO† | 10.0 | 207 | | | | 10 | 18 | 23 | 29 |
| I. sp. UW-330 | not done | 207 | 99 | | | | | | |

*As per cent of total sterol, CHOL = cholesterol; BRASS = brassicasterol; 24MC = 24-methylcholesterol; 24EC-5,22 = 24-ethylcholesta-5,22-dienol; 24EC = 24-ethylcholesterol; MPAV = methylpavlovol; EPI = epibrassicasterol.

†Also contains small amounts of 4-methyl and dihydroxy sterols.

sterol, which was present almost exclusively in the free sterol fraction. This sterol appeared by capillary gas chromatography ($RR_t$ 1.10 relative to cholesterol) and mass spectroscopy ($m/z$ 398, $[M]^+$ additional peaks at $m/z$ 383, 380, 365, 355, 337, 300, 271, 255, 229 and 213) to be either brassicasterol or its C-24 isomer. Previous work showed that in T-ISO and C-ISO, this sterol was the C-24 $\beta$-isomer, brassicasterol. Use of an HPLC column [20] that separates brassicasterol ($RR_t$ 0.84) and epibrassicasterol ($RR_t$ 0.64) on strains BL-ISO and LB-1292 showed that they contained epibrassicasterol. Each of the strains containing epibrassicasterol (Table 1) had a total sterol content of 5–6 $fg\,cell^{-1}$, which was much lower than other strains tested. Each of these strains also contained long chain alkenones ($C_{37}$–$C_{39}$) which occur in many but not all members of the Isochrysidales [17].

All strains of *Isochrysis* examined previously were shown to contain long chain alkenones [17, 21, 22]. The alkenone composition of the *Isochrysis* strains containing brassicasterol is seen in Table 2. The principal components are $C_{37}$-alken-2-ones and $C_{38}$-alken-3-ones with two, three or four double bonds. Structures and double bond locations have been established for these compounds isolated from several strains of *Isochrysis* [17, 21, 22]. Traces of $C_{39}$-alkenones also were present. The alkenone compositions of strains LB-1292 and BL-ISO were quite similar to that reported for *Isochrysis galbana* I [17].

Strains T-ISO and C-ISO contain brassicasterol at 21–31 $fg\,cell^{-1}$, and the same four principal alkenones as BL-ISO and LB-1292, but are distinguished by significantly higher per cents of 37:2, smaller per cents of 38:3 and the virtual absence of 37:4, as compared to the other two. Although precise quantitation was not possible, it was clear that alkenones were present in larger quantities than sterols (10× or more) and were present in larger quantities in strains T-ISO and C-ISO than in LB-1292 and BL-ISO.

Strains UW-330 and ISO contained no detectable brassicasterol, epibrassicasterol or alkenones. Strain

Table 2. Alkenones in *Isochrysis* sp.*

| Alken-2-ones | Strain LB-1292 | BL-ISO | T-ISO | C-ISO |
|---|---|---|---|---|
| 37:4 | 3 | 3 | | |
| 37:3 | 55 | 58 | 42 | 38 |
| 37:2 | 6 | 5 | 30 | 28 |
| Alken-3-ones | | | | |
| 38:3 | 20 | 20 | 5 | 7 |
| 38:2 | 13 | 10 | 18 | 26 |

*As per cent of total alkenone.

UW-330 contained cholesterol exclusively in the free sterol fraction as the only significant sterol. Its cellular sterol concentration (207 $fg\,cell^{-1}$) was much greater than in the strains containing brassicasterol or epibrassicasterol (Table 1). The presence of cholesterol rather than brassicasterol or epibrassicasterol, and the absence of alkenones raises the question of whether this alga belongs in the genus *Isochrysis*. The Milford strain ISO also contained a large quantity of sterol (207 $fg\,cell^{-1}$) which was composed of $\Delta^5$-sterols, 4-methyl sterols and dihydroxysterols found in the ester, free and glycoside fractions as seen in *Pavlova* species [16, 23–25].

The strain BL-ISO (CCMP1323) originated from the type strain of *I. galbana* from the Plymouth collection; we obtained it from the Provasoli-Guillard Center for the Culture of Marine Phytoplankton just prior to conducting this study, and its composition is consistent with previous studies of *I. galbana* [15–18]. The Milford ISO strain had a sterol composition considerably different from BL-ISO, but quite similar to that of *Pavlova lutheri* [24]. We know from light microscope observations and SEM (Wikfors, unpublished results) that the Milford 'ISO' is not *P. lutheri*. However, because of the presence of an abbreviated haptonema in the Milford ISO, cultures

which were separated from the parent ISO strain (between 35 and five years ago) for particular research projects and maintained in parallel were examined. All contained a sterol composition of $\Delta^5$-sterols and dihydroxysterols similar to that seen for ISO in Table 1. As this algal strain has been used in many published studies from the Milford Laboratory over many years, implications of its unique biochemistry may be considerable.

Two stains of *Isochrysis* analysed in the present study (UW-330, ISO) were very different from the other four in that they had a much larger quantity of sterol, a different type of sterol, and did not contain long chain alkenones. They should be re-examined taxonomically. The Milford ISO strain will be re-named M-ISO in the Milford Collection and be referred to as *Isochrysis* sp. until such taxonomic studies are conducted. The other four strains contained brassicasterol or epibrassicasterol as their only significant sterol, but two strains (T-ISO and C-ISO) could be distinguished from the other two on the basis of their sterol and their alkenone composition (Table 2). $C_{28}$- and $C_{29}$-$\Delta^{5,22}$-sterols are the principal sterols in nearly all species of Prymnesiophyceae studied [26]. However, four species contain cholesterol as the principal sterol [17], so the presence of cholesterol in UW-330 is not unprecedented in the Prymnesiophyceae.

This report confirms that *Isochrysis* strains differ in sterol and alkenone composition, and suggest that these data may be useful in taxonomic studies of this important group of phytoplankton, and also may be responsible in part for differences in nutritional value of the strains to bivalves and other invertebrates.

## EXPERIMENTAL

*Algal culture.* All cultures were grown axenically (although UW-330 did contain a bacterial contaminant) in enriched natural seawater 'E' formulation in carboys and harvested in the stationary phase as described previously [24, 27]. Sources of the cultures used are as follows: UW-330 was from the University of Washington culture collection, LB-1292 was from the University of Texas culture collection, T-ISO, C-ISO, and ISO were from the Milford Laboratory culture collection, and BL-ISO was from Provasoli-Guillard Center for the Culture of Marine Phytoplankton (their strain designation CCMP1323). Stationary phase cultures were centrifuged, frozen in cold MeOH (−25°) and lyophilized as described previously [24], and stored in a freezer until analysed. Cells were enumerated microscopically, and dry weights were determined as described previously [9].

*Lipid analysis.* Lyophilized samples (0.5–1.0 g) were extracted overnight in a Soxhlet extraction with $CHCl_3$–MeOH (2:1) and the crude lipid was partitioned into ester, free alcohol and glycoside frs by Biosil A CC. Ester and glycoside were base and acid hydrolysed, respectively, and sterol analyses were conducted on all frs [23] by capillary gas chromatography on a Varian 3500 gas chromatograph equipped with a 30 m × 0.25 SPB-1 column. Sterols were identified and quantified by comparison with a cholesterol standard. Mass spectra of sterols were obtained with a Finnigan-MAT Model GC-MS equipped with a 30 m × 0.25 mm fused silica capillary column coated with a 0.25 mm film of DB-1. Electron impact mass spectra were measured at 70 eV and an ionization temp. of 150°. Data were recorded and analysed with an Incos Data System. Alkenones were identified by their $RR_t$ on gas chromatography [17] and by electron impact and chemical ionization MS.

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